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Postbinding defects of insulin action in human adipocytes from uremic patients

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Postbinding defects of insulin action in human adipocytes from uremic patients. It is now well established that longstanding human uremia is associated with impaired in vivo insulin action on glucose utilization of peripheral target tissues. In an attempt to define the cellular basis of the uremic insulin resistance we studied insulin action in adipocytes from eight patients with undialyzed chronic uremia and from eight matched healthy controls. (^{125}I)-Insulin binding to fat cells from uremic patients was normal. In contrast (^{14}C)-D-glucose transport exhibited decreased sensitivity to insulin. The concentrations of insulin that elicited half-maximal response was 422 ± 95 pmoles/liter in uremic patients and 179 ± 38 pmoles/liter in normal subjects ($P < 0.01$). The noninsulin- and the maximal insulin-stimulated glucose transport of adipocytes from uremic patients was normal. (^{14}C)-D-glucose conversion to total lipids was also measured in these cells in the absence and presence of various insulin concentrations. Similar to the findings in transport studies the lipogenesis of fat cells from uremic patients had depressed sensitivity to insulin (half-maximal stimulation at 38 ± 8 pmoles/liter in uremic patients and at 11 ± 3 pmoles/liter in normal subjects, $P < 0.01$) with unchanged noninsulin and maximal insulin-stimulated lipogenesis. Taken together these results suggest that the insulin resistance of adipocytes from patients with chronic uremia may be accounted for primarily by postbinding defects localized to glucose transport and metabolism.

Anomalies de l'action de l'insuline en aval de la liaison dans des adipocytes humains provenant de malades en urémie. Il est maintenant bien établi que l'urémie humaine au long cours s'associe à une altération de l'action in vivo de l'insuline sur l'utilisation du glucose par les tissus cibles périphériques. Dans une tentative de définir la base cellulaire de la résistance urémique à l'insuline, nous avons étudié l'action de l'insuline dans les adipocytes de huit malades en urémie chronique, non dialysés, et de huit contrôles sains appariés. La liaison de l'(^{125}I)-insuline à des adipocytes de malades urémiques était normale. A l'opposé, le transport du (^{14}C)-D-glucose présentait une sensibilité à l'insuline diminuée. Les concentrations d'insuline entrainant la moitié de la réponse maximale étaient de 422 ± 95 pmoles/litre chez les malades urémiques, et 179 ± 38 pmoles/litre chez les normaux ($P < 0{,}01$). Le transport de glucose maximum stimulé par l'insuline ou non stimulé par l'insuline d'apidocytes d'urémiques était normal. La conversion du (^{14}C)-D-glucose en lipides a été aussi mesurée dans ces cellules, en l'absence ou en présence de concentrations variées d'insuline. Comme pour les résultats des études de transport, la lipogenèse des cellules graisseuses des malades urémiques avait une sensibilité diminuée à l'insuline (stimulation demi-maximale pour 38 ± 8 pmoles/litre chez les malades urémiques, et pour 11 ± 3 pmoles/litre chez les normaux $P < 0{,}01$), avec une lipogenèse non insulinique ou stimulée au maximum par l'insuline inchangée. Pris dans leur ensemble, ces résultats suggèrent que la résistance à l'insuline d'adipocytes de malades en urémie chronique pourrait être due primitivement à des anomalies en aval de la liaison, au niveau du transport et du métabolisme du glucose.

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Chronic uremia is often associated with carbohydrate intolerance [1]. In studies using glucose tolerance tests or the glucose clamp technique, it has been shown that endogenous insulin secretion is normal in most cases of chronic uremia [1, 2]. Numerous experiments based on the forearm technique, intravenous insulin or tolbutamide tests [for review, see 1, 3–5] and the euglycemic clamp technique [6, 7] demonstrate that the glucose intolerance of uremic patients is due primarily to insulin resistance of peripheral tissues. Moreover, studies of the in vivo turnover of tritiated glucose have shown that the liver of uremic humans retains normal sensitivity to insulin at least with respect to the inhibitory effect on hepatic glucose release [6, 7].

The purpose of our present study was to define and examine the cellular mechanisms behind the insulin resistance of peripheral tissue from uremic humans. In so doing, we measured (^{125}-I)-insulin binding and noninsulin and insulin-stimulated (^{14}-C)-D-glucose transport and metabolism in isolated human adipocytes from healthy control subjects and from uremic patients not yet on chronic dialysis treatment.

Methods

Subjects

Eight adult, ambulatory nondiabetic uremic patients were studied and compared with sex-, age-, and weight-matched healthy volunteers. Pertinent clinical data for the patients appear in Table 1. All patients with uremia exhibited significant in vivo insulin resistance of glucose uptake into peripheral tissue as judged by studies using the euglycemic clamp at several steady-state plasma insulin concentrations (unpublished data). None of the patients presented gastrointestinal symptoms or clinical evidence of malnutrition. The patients were taking the following medication: beta-adrenergic blockers (patients numbered 2, 5, 6, and 8), hydralazine (patient 2), $NaHCO_3$ (patient 3), NaCl (patient 4), and 1,25 dihydrocholecalciferol (patient 8). No medication was given for the last 36 hr before the examination. All patients performed an oral glucose tolerance

Table 1. Clinical and laboratory data of patients

Patient no.	Sex	Age *years*	Obesity index	Renal disease	Plasma		Serum		
					Insulin *μU/ml*	Glucose *mmoles/liter*	Creatinine *μmoles/liter*	Urea *mmoles/liter*	Total CO_2 *mmoles/liter*
1	M	52	1.04	Chronic glomerulonephritis	19	6.5	884	38.6	15
2	M	53	0.94	Polycystic kidney disease	8	4.2	887	28.5	15
3	F	55	1.00	Polycystic kidney disease	11	5.1	766	30.6	19
4	M	58	0.97	Chronic interstitial nephritis (analgesic)	22	4.8	929	18.7	16
5	F	55	0.92	Chronic interstitial nephritis (analgesic)	12	4.6	678	46.2	25
6	F	31	0.94	Chronic glomerulonephritis	13	4.6	833	23.6	17
7	F	48	1.35	Chronic interstitial nephritis (not classified)	15	4.8	808	20.8	20
8	M	43	1.15	Chronic glomerulonephritis	11	5.3	559	18.8	22
Mean		49	1.04		14	5.0	793	28.2	19
SEM		0.2	0.05		2	0.2	55	3.6	1

Table 2. Average daily intake (mean ± 1 SEM) of energy and nutrients during the last 3 days before the study

	Uremic patients	Normal subjects
Energy, *kj*	6863 ± 690	7068 ± 669
Digestible carbohydrate, *g*	181 ± 17 (44 energy%)	175 ± 13 (40 energy%)
Nondigestible carbohydrate, *g*	23 ± 2	21 ± 2
Protein, *g*	48 ± 5 (12 energy%)	71 ± 6 (16 energy%)
Fat, *g*	80 ± 11 (44 energy%)	87 ± 9 (44 energy%)

test (1 g of glucose/kg body wt). Patient 2 had a 2-hr value of plasma glucose at 11.6 mmoles/liter and patient 7 had a 2-hr value at 9.8 mmoles/liter, whereas the rest of the group all had 2-hr values below 7.5 mmoles/liter.

Four healthy males and four healthy females served as control subjects with a mean age of 44 years (range, 58 to 26 years) while the mean obesity index was 1.05 (range, 0.85 to 1.15). In normal subjects the average fasting plasma concentrations of glucose and insulin were 4.8 mmoles/liter (range, 4.2 to 5.4) and 10 μU/ml (2 to 18), respectively. Each participant in the study recorded the daily intake of all food and drink 3 days before the investigation. The dietary composition was calculated according to dietary tables, and the average results are given in Table 2. Informed consent was obtained from all subjects after the purpose and the potential risks of the study were explained carefully. The study protocol was approved by the local ethical committee. All tests were performed in the postabsorptive state at 7 A.M. following a 12-hr overnight fast.

Chemicals

Human albumin was obtained from Behringswerke, Marburg, West Germany. Collagenase from Clostridium histolyticum, 213 U/mg was obtained from Worthington Biochemical Corporation (Freehold, New Jersey, USA). (^{125}I) Monoiodoinsulin with the labeled iodine in tyrosine A_{14} (sp act about 250 μCi/μg) was donated generously by NOVO Research Institute, Copenhagen, Denmark. D-U-(^{14}C) glucose (sp act 284 μCi/mmoles) were obtained from the Radiochemical Centre, Amersham, England. Tissue and cells were suspended in Hepes buffer (10 mmoles/liter, pH 7.4 at 37°C) [8].

Insulin receptor binding

Adipose tissue samples (about 10 g) were obtained by open biopsy from the upper one-fourth of the right gluteal region after a square field had been anesthetized with an epidermal injection of 1% lidocaine without epinephrine. Details concerning fat cell isolation as well as the determination of fat cell size and number have been published previously [8]. The mean fat cell diameter was 91 ± 11 μm in uremic patients and 95 ± 12 μm in control subjects ($t^2 > 0.1$). Insulin binding to fat cells (about 10^5 cells/ml of cell suspension) was measured in a Hepes buffer at 37°C, after a 60-min incubation with tyrosine-A_{14}-labeled (^{125}I) insulin with or without increasing concentrations of unlabeled insulin [8]. Cell-associated radioactivity in the presence of 100 μmoles/liter unlabeled insulin (nonspecific binding) averaged 2% of the total binding. Specific insulin binding to adipocytes was expressed per 30 cm^2 of surface area per milliliter.

Glucose transport

The initial glucose transport rate was measured by a modification of our previously described method [9]. All studies were carried out at 37°C. Adipocyte suspension (40 μl) with a volume fraction of 0.4 (about 6×10^5 cells/ml) were placed in polypropylene tubes and preincubated with or without insulin for 45 min. Tracer D-U-(^{14}C)-glucose [12 μl (0.24 μCi); final glucose concentration 20 μmoles/liter] was added at time zero and uptake was determined after 10 sec by adding 3 ml of phloretin (0.3 mmoles/liter). Silicone oil (0.8 ml, 0.99 g/ml) was layered on the top, and the tubes were spun within 2 min at ×2500*g*. Cells were collected from the top of the oil and placed in scintillation vials with 5 ml of scintillation fluid. Extracellular trapped radioactivity was estimated by adding phloretin before the tracer. All values were corrected for extracellular trapped radioactivity which averaged 25% of the tracer glucose uptake after 10 sec in the absence of insulin. The initial uptake of tracer glucose (20 μmoles/liter) is linear from 2.5 sec to 5 min [10]. The

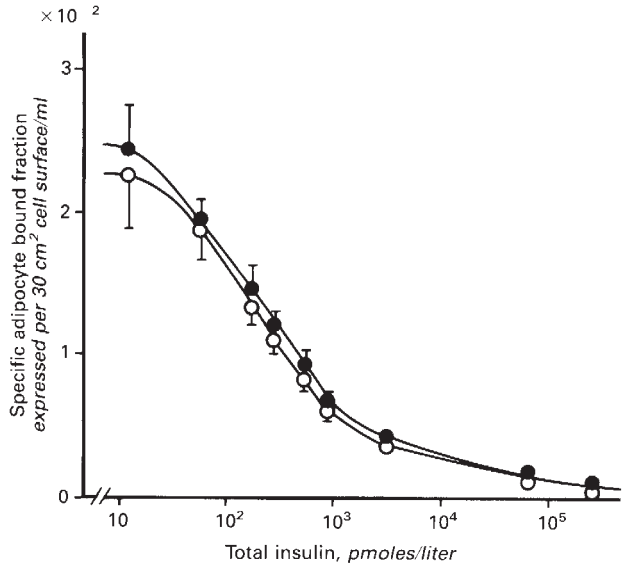

Fig. 1. *Insulin binding to fat cells.* Adipocytes from eight uremic patients (●) and eight normal subjects (○) were incubated with 15 pmoles/liter (^{125}I)-insulin at 37°C for 1 hr in the absence or presence of unlabeled insulin at the indicated concentrations (mean ± 1 SEM).

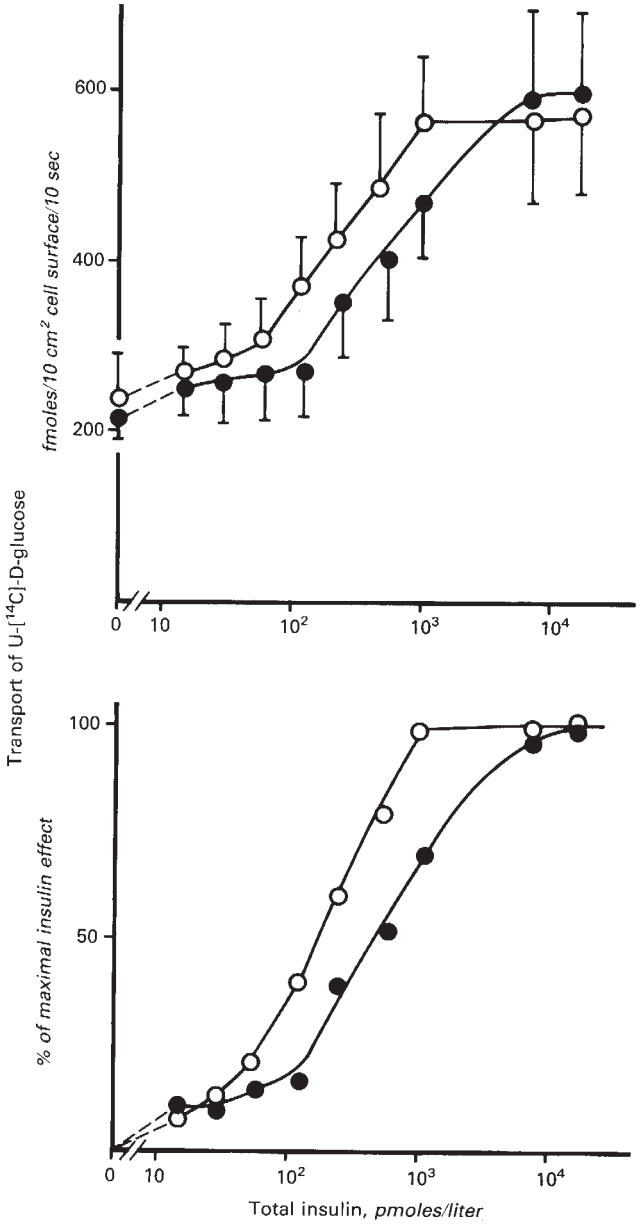

Fig. 2. *Glucose transport of fat cells. Upper panel:* Adipocytes from eight uremic patients (●) and eight normal subjects (○) were preincubated with or without insulin for 45 min at 37°C. The initial transport rate was then measured during the first 10 sec after (^{14}C)-D-glucose had been added to reach a final concentration of 20 μmoles/liter (mean ± 1 SEM). *Lower panel:* Glucose transport data are expressed as the percentage of the response to insulin in maximally effective concentrations.

coefficient of variation for uptake of (^{14}C)-D-U-glucose was 0.09 after 10 sec.

Lipogenesis

Lipogenesis was measured as the conversion of D-U-(^{14}C) glucose to (^{14}C)-total lipids [11]. Isolated adipocytes (about 7 × 10^4 cells/ml) were prepared in a 10 mmoles/liter Hepes buffer containing 0.5 mmoles/liter glucose. The cells were preincubated for 45 min at 37°C with or without insulin in increasing concentrations. Then 0.4 μCi D-U-(^{14}C) glucose was added to each tube (final glucose concentration; 0.5 mmoles/liter) and the incubation was continued for 90 min followed by the addition of H_2SO_4. A Dole extraction was also performed [11]. A sample for liquid scintillation counting was taken from the upper phase. (^{14}C)-labeled total lipids were present in an average amount of 16% of the noninsulin-stimulated lipogenesis when incubations were performed in the absence of fat cells (blank values). All values for the fat cell producing total lipids were corrected for the individual blank value.

Analytical methods

Plasma glucose was analyzed with a glucose dehydrogenase method (Merck enzymatic kit). Plasma insulin was measured with a RIA technique [12].

Statistical methods

In the text, table and figure data are given as the mean ± 1 SEM. Significant differences between the groups were assessed by the Mann Whitney test. In correlation studies the Spearman test was used.

Results

The uremic condition did not influence the binding of (^{125}I)-insulin or the ability of unlabeled insulin to compete for the binding over a concentration range of 0.05 to 245 nmoles/liter (Fig. 1). Scatchard analysis (not shown) of competitive binding data did not show apparent differences in the affinity or the number of receptors per adipocyte between uremic and control subjects. There were no significant correlations between insulin binding variables and the fasting plasma (or serum) concentrations of insulin, creatinine, urea or carbon dioxide.

When (^{14}C)-D-glucose transport was measured in the absence or the presence of maximally effective insulin concentration adipocytes from uremic patients responded similarly to adipocytes from healthy volunteers (Fig. 2). However, the

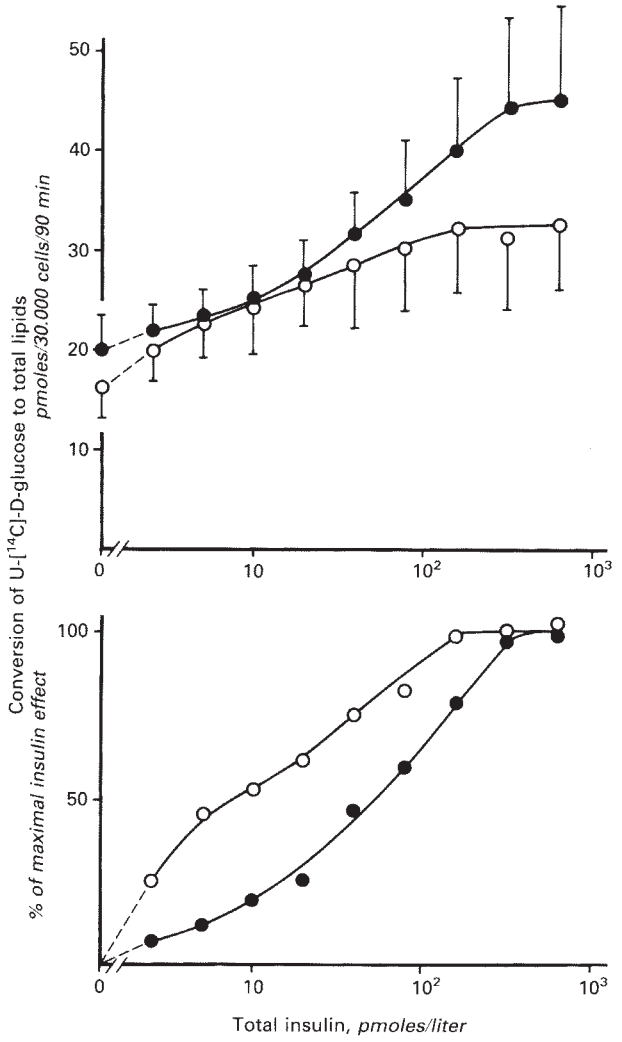

Fig. 3. *Lipogenesis of fat cells. Upper panel:* Adipocytes from eight uremic patients (●) and eight normal subjects (○) were preincubated with or without insulin in the indicated concentrations for 45 min at 37°C. Then (^{14}C)-D-glucose was added (total glucose concentration in the final preparation was 0.5 mmoles/liter) and the incubation was terminated after 90 min (mean ± 1 SEM). *Lower panel:* Lipogenesis data are expressed as the percentage of the response to insulin in maximally effective concentrations.

insulin-dose response relationship of glucose transport was altered by uremia. In patients with renal failure the dose-response curve for insulin-stimulated glucose uptake was shifted markedly to the right (Fig. 2) suggesting an impaired sensitivity to insulin. For example, the concentrations of insulin that elicited half-maximal effect were 422 ± 95 pmoles/liter in uremic patients and 179 ± 38 pmoles/liter in healthy subjects ($P < 0.01$).

The effect of uremia on glucose metabolism by human adipocytes was studied by measuring (^{14}C)-D-glucose conversion to total lipids (Fig. 3). Both noninsulin- and maximal insulin-stimulated lipogenesis were normal in uremic cells. However, consistent with our observations in glucose transport studies, the insulin concentrations giving half-maximal lipogenesis were significantly higher in adipocytes from uremic patients (38 ± 8 pmoles/liter in uremic patients versus 11 ± 3 pmoles/liter in normal subjects, $P < 0.01$). No significant correlations could be found between values of noninsulin stimulated, half-maximal stimulated and maximal insulin-stimulated glucose transport or metabolism and fasting plasma (serum) concentrations of insulin, creatinine, urea, or carbon dioxide.

Discussion

Our present report provides evidence of the cellular mechanisms underlying the insulin resistance of glucose utilization of peripheral tissue in patients with chronic renal disease. We are well aware that adipose tissue is not the major organ among peripheral tissues with respect to insulin-enhanced glucose removal [13]. Adipocytes are, however, peripherally fixed cells which are accessible for in vitro studies of insulin binding and postbinding events [14]. Our results indicate that long-term uremia has no significant impact on adipocyte insulin receptor binding. Previous investigations of blood cell insulin receptors in human uremia have shown normal monocyte binding and decreased or normal erythrocyte binding [7, 15–18]. Additionally, studies of adipocyte insulin binding from uremic rats [19, 20] are consistent with our finding in humans. Taken together, the available literature suggests that the insulin resistance in human uremia does occur in the absence of altered insulin receptor binding.

Uremic defects in adipocyte insulin action were found in the insulin-dose response studies of glucose transport as well as glucose metabolism. Both cellular functions were in uremic cells characterized by impaired sensitivity to submaximal insulin stimulation but unaltered basal and maximal responses. Whether the depressed insulin sensitivity of lipogenesis in uremia represents intracellular defects of glucose metabolism per se or is secondary to the impaired glucose transport cannot be decided from the present study.

In comparison, studies of adipocytes from uremic rats have shown that acute (24 hr) uremia [19] has no effect on noninsulin- or insulin-enhanced glucose oxidation whereas chronic uremia (14 days) caused a significant decrease of basal and maximal glucose transport and metabolism [20]. In both studies the sensitivity of the measured pathways to insulin was unaffected compared with the normal state. The disparity between the study by Maloff, McCaleb, and Lockwood [20] and the present investigation obviously may be related to the different protocols (species, degree and duration of uremia, and so forth).

The regulatory mediator(s) of cellular insulin resistance in uremia is unknown and in our study we failed to show any correlation between insulin concentrations causing half-maximal responses of glucose transport or metabolism and plasma (serum) insulin, creatinine, urea, or carbon dioxide. Recent studies in animals suggest, however, that the altered postbinding effects of insulin in uremia may in part arise from circulating factors not yet identified [20, 21].

The in vivo studies of the insulin resistance of human uremia using the euglycemic clamp technique at more steady-state plasma insulin levels have demonstrated both a rightward shift of insulin dose-response curves and a decreased maximal insulin responsiveness of the glucose disposal to peripheral tissues [6, 7]. Under the insulin clamp study conditions the major site of glucose uptake is muscle. Unfortunately, there is no available technique which allows measurement of cellular

insulin action in human muscle, but, provided our finding of normal adipocyte insulin receptor binding can be extrapolated to muscles, the clamp results are most compatible with postbinding defects in insulin mediated in vivo glucose utilization. Finally, because we could not find depression of maximal adipocyte glucose metabolism in vitro, the in vivo finding of impaired maximal responsiveness of the glucose disposal [6, 7] may reflect additional defective postbinding steps of insulin action in skeletal muscles.

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